Determination of Rutin in Buckwheat Leaf Meal and Other Plant Materials

Absorptiometric Method

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The present gravimetric method for rutin is slow, and is not precise when the rutin concentration is small. In the proposed method, rutin is extracted with ethanol and isolated from other plant pigments by a phase transfer as the aluminum chloride complex. The rutin thus isolated is estimated by the absorbance measured at 416 mμ. The reliability was tested by replicate analyses and by determination of rutin added to both rutin-containing buckwheat leaf meal and marc. With buckwheat leaf meals, the absorptiometric method gave generally higher rutin contents than the gravimetric method, and the divergence increased at the lower rutin levels. A few analyses by both methods were made on leaves of tobacco, wild cherry, common elder, and *Eucalyptus macrorrhyncha*, and on buds of *Sophora japonica*. The absorptiometric method requires fewer operations and only about one tenth the time necessary for the gravimetric method without sacrificing accuracy or precision.

THE discovery that rutin (3-rhamnoglucoside of 5,7,3′,4′-tetrahydroxyflavonol) is useful for the correction of capillary fragility and permeability in man (*1, 5, 13*) led to the evaluation of various plant materials as commercial sources of this compound. The first domestic commercial source of rutin was buckwheat. Several publications (*3, 4, 6, 8, 10*) have dealt with the preferred species, optimum planting and harvesting conditions, preparation of leaf meal, and large scale extraction and isolation of rutin. More recently, *Sophora japonica* (*2*) and *Euclayptus macrorrhyncha* (*12*) have become important commercial sources.

The method now used for determination of rutin in plant materials is a gravimetric extraction-isolation technique (*7*) that requires about 10 days. Because this method is slow, includes a number of operations, and is unreliable when applied to materials of low rutin content, the following procedure has been developed. The proposed procedure involves the extraction of rutin from the plant material, isolation of rutin by a phase transfer as a colored complex (*11*), and subsequent estimation of the concentration by absorptiometry. The proposed method offers a great reduction in time (approximately 8 hours being required for complete analysis) and greater precision and accuracy than the gravimetric method. This reduction in time will make it possible to use the method for control purposes during the manufacturing process.

METHOD

Equipment and Reagents. Absolute ethanol; benzene-denatured absolute ethanol (formula 12A) is suitable.

Aluminum chloride, 0.1 *M*.

Isoamyl alcohol, boiling point 129–132° C.

Smalley extraction-tube assembly, ground joints, 125-ml. ask.

International centrifuge, Size 1, Type SB, with head and trunnion carriers suitable for carrying 125-ml. Squibb separatory-funnel type centrifuge tubes.

Beckman Model DU spectrophotometer, with 1-cm. absorption cells.

Determination of Moisture (7). The sample of meal is ground to approximately 200 mesh, and 10-gram samples of the ground meal are dried at 110° C. for 6 to 8 hours. The loss in weight is calculated as per cent moisture.

Extraction of Ground Meal. A 2-gram sample of the ground meal is distributed evenly over the surface of a piece of absorbent cotton, approximately 4 × 2 × 0.25 inches. The cotton is rolled along its major axis and placed in a Smalley extractor, the bottom of which contains a small plug of cotton. The extractor is fitted to the extraction flask, which contains a few glass beads, and approximately 75 ml. of absolute ethanol is poured through the cotton in the extractor. (Ethanol is preferred to methanol because ethanol dissolves less nonrutin colored plant material.) The sample is extracted for 6 hours. The cooled extract is diluted to 250 ml. with isoamyl alcohol.

Isolation of Rutin. A 20-ml. aliquot of the isoamyl alcohol solution is placed in a 125-ml. Squibb separatory funnel and extracted with three 25-ml. portions of 0.1 *M* aluminum chloride. During this operation, the rutin passes into the aqueous phase as the yellow rutin-aluminum chloride complex, leaving the other extracted plant pigments in the isoamyl alcohol layer. After each shake-out, the solvents are separated by centrifugation, and the lower aqueous layer is run off into a 250-ml. volumetric flask. The combined aqueous extracts are diluted to 250 ml. with distilled water.

Absorptiometry. The rutin-aluminum chloride complex has an absorption maximum at 416 mμ. The absorbance of the solution at its final dilution is determined with a Beckman DU spectrophotometer at 416 mμ, versus a correspondingly diluted aluminum chloride blank, using 1-cm. cells. No appreciable absorption has been observed in blank analyses of the alcohol reagents or of extracts from the cotton, alone or in combination with the aluminum chloride solution. Appropriate dilutions are made to maintain the absorbance between 0.2 and 0.8. Through this range the solutions follow Beer's law accurately. The weights and dilutions given are suitable for materials having rutin concentrations of 1 to 4%. The rutin complex is stable, and the absorbance may be determined almost immediately and remains unchanged for at least 2 hours. Glass or interference filter absorptiometers isolating the 416-mμ region can probably be used,

but for each individual instrument the absorptivity factor would have to be established.

Replicate analyses of pure rutin (rutin·3H$_2$O) (9) have established its absorptivity, a, as 30.7 under the conditions described. The per cent rutin trihydrate may be calculated from the following equation.

$$\% \text{ rutin.3H}_2\text{O} = \frac{A}{ab} \times \frac{V_f}{1000} \times \frac{V_e}{v} \times \frac{100}{w}$$

where A = abc

A = absorbance $\left(\log \frac{1}{\text{transmittance}}\right)$

a = absorptivity (A/bc)

b = cell length, cm.

c = concentration of rutin, grams per liter

V_f = final volume of rutin-complex solution, ml.

V_e = volume of extract diluted with isoamyl alcohol, ml.

v = volume of aliquot taken, ml.

w = dry weight of sample, grams

Under the conditions in this procedure, this equation reduces to

$$\% \text{ rutin.3H}_2\text{O} = \frac{A}{w} \times 10.2$$

RESULTS AND DISCUSSION

The procedure described has been used in this laboratory for more than a year, during which time numerous comparisons have been made with the gravimetric method. Table I lists twelve such comparisons. Data given for each method are the average of at least duplicates. In most cases in which the rutin content was 3% or more, the two procedures agreed to within ±10%. When the rutin content was low, however, the two methods did not agree, in some cases the proposed method being as much as 28% higher. With samples of low rutin content, the gravimetric method is complicated by slowness of precipitation—sometimes several crops of crystals must be collected before complete precipitation can be assumed.

Table I. Rutin Content of Buckwheat Leaf Meals

Sample No.	Rutin.3H$_2$O, %		Difference[a], %
	Absorptiometric	Gravimetric	
F 724	6.28	5.69	− 9.4
F 735	5.33	5.01	− 6.0
F 720	4.89	5.16	+ 5.5
F 727	4.40	4.26	− 3.2
F 756	3.98	3.93	− 1.3
F 785	3.91	3.85	− 1.5
F 746	3.35	3.48	+ 3.9
F 777	3.30	2.74	−17.0
F 759	3.09	2.85	− 7.8
F 767	2.94	2.21	−24.8
F 770	2.50	1.80	−28.0
F 764	2.44	1.83	−25.0

[a] Difference, % = $\dfrac{\text{gravimetric \% − absorptiometric \%}}{\text{absorptiometric \%}} \times 100$.

To establish the degree of precision that can be expected from the proposed method, a single sample was analyzed nineteen times. Table II summarizes the results of these replicate analyses.

Because the proposed method was occasionally at variance with the gravimetric method and recovery experiments were not performed by the gravimetric method, two sets of recovery experiments were made by the absorptiometric method. One set, with

Table II. Replicate Analyses of Single Buckwheat Leaf Meal Sample

No. of Analyses	Rutin.3H$_2$O, %			Standard Deviation[a]
	Min.	Av.	Max.	
19	4.42	4.52	4.68	0.08

[a] Standard deviation, $s = \sqrt{\dfrac{\Sigma(x - \bar{x})^2}{n - 1}}$

quadruplicate samples, attempted recovery of 2.65% rutin added to a sample containing 2.17% rutin. Recoveries were 100 ± 0.8%. The purpose of the other experiment was the recovery of rutin in the ranges of 0.5, 0.9, 1.7, and 3.7% from buckwheat leaf meal marc. At the 3.7% level, recovery was 100 ±1%. At the lower levels, recovery ranged between 94.5 and 100%.

Application to Other Plant Sources. The proposed method was applied to the analysis of other plant sources. Table III lists these sources, and gives results by the absorptiometric and gravimetric methods. Again the agreement was satisfactory when the concentration was high and poor when low. An important consideration if this method is to be used for screening plant materials is the limited specificity of the aluminum chloride. Aluminum chloride is not a specific reagent for rutin; other flavonols give similarly colored complexes. The location of the absorption maximum helps in evaluating the validity of the analysis, because other flavonol-aluminum complexes have their maxima at different wave lengths. Examination of the absorption spectra in the 350- to 450-mμ region will indicate interferences when they are present.

Table III. Rutin Content of Plant Samples Other Than Buckwheat

Species	Type of Material	Rutin.3H$_2$O, %	
		Absorptiometric	Gravimetric
Nicotiana tabacum	Flue-cured leaves	0.97	0.21
Prunus melanocarpa	Dried leaves	2.09	1.66
Sophora japonica	Dried flower buds	17.3	15.9
Sambucus canadensis	Dried leaves	3.80[a]	3.45
	Dried immature blossoms	4.93[a]	5.18
	Dried mature blossoms	3.90[a]	3.00
Eucalyptus macrorrhyncha	Fresh green leaves	9.20[a]	8.95

[a] Analysis performed on aliquots of extracts prepared for gravimetric procedure.

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